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# Alanine aminotransferase predicts coronary heart disease events: A 10-year follow-up of the Hoorn Study☆

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**Abstract**

Alanine aminotransferase (ALT) is a marker of non-alcoholic fatty liver disease (NAFLD) and predicts incident type 2 diabetes mellitus (DM2). Recently, ALT was shown to be also associated with endothelial dysfunction and carotid atherosclerosis. We studied the predictive value of ALT for all-cause mortality, incident cardiovascular disease (CVD) and coronary heart disease (CHD) events in a population-based cohort of Caucasian men and women aged 50–75 years, at baseline. The 10-year risk of all-cause mortality, fatal and non-fatal CVD and CHD events in relation to ALT was assessed in 1439 subjects participating in the Hoorn Study, using Cox survival analysis. Subjects with prevalent CVD/CHD and missing data were excluded. As compared with the first tertile, the age- and sex-adjusted hazard ratios (95% confidence intervals) for all-cause mortality, CVD events and CHD events were 1.30 (0.92–1.83), 1.40 (1.09–1.81) and 2.04 (1.35–3.10), respectively, for subjects in the upper tertile of ALT. After adjustment for components of the metabolic syndrome and traditional risk factors, the association of ALT and CHD events remained significant for subjects in the third relative to those in the first tertile, with a hazard ratio of 1.88 (1.21–2.92) and 1.75 (1.12–2.73), respectively. In conclusion, the predictive value of ALT for coronary events, seems independent of traditional risk factors and the features of the metabolic syndrome in a population-based cohort. Further studies should confirm these findings and elucidate the pathophysiological mechanisms.


*Keywords:* Alanine aminotransferase; Epidemiology; Coronary heart disease events

## 1. Introduction

Recently, the role of non-alcoholic fatty liver disease (NAFLD) in the pathogenesis of type 2 diabetes mellitus (DM2) has gained much interest. Several studies have demonstrated that NAFLD is associated with the components of the metabolic syndrome (MetS) and DM2 [1,2], and fatty liver is considered to be the hepatic component of the MetS [3,4]. Circulating concentrations of the liver transaminases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) have been used as markers of NAFLD. Of these two liver enzymes, ALT appears to be the best marker of liver fat accumulation and is positively correlated with liver fat measured by magnetic resonance (MR) proton spectroscopy [5]. In cross-sectional and prospective studies ALT is associated with DM2 [6,7]. Collectively, these data suggest that hepatic steatosis might play a role in the pathogenesis of DM2, most probably by contributing to the development of hepatic insulin resistance resulting, among others, in increased

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hepatic gluconeogenesis and overproduction of triglyceride-rich lipoproteins. Subjects with features of the MetS are not only at increased risk of DM2, but also at risk of developing cardiovascular (CVD) and coronary heart disease (CHD) [8].

In view of these observations, and the recently described associations between both NALFD or its marker ALT and vascular structural and functional properties [9,10], we studied the predictive value of ALT for all-cause mortality, CVD and CHD events in a population-based study of diabetes and related complications in Caucasian men and women aged 50–75 years, at baseline.

## 2. Methods

### 2.1. Study population

Subjects were participants in the Hoorn Study, a prospective population-based cohort study of glucose metabolism and diabetes complications. The study population and research design have been described in detail previously [11]. In short, in 1989, a random sample of all men and women aged 50–75 was taken from the municipal registry of the medium-sized town of Hoorn in The Netherlands. Of the 3552 individuals, who where invited to take part in the study, 2540 agreed to participate (71.5%). Baseline data, including a 75 g oral glucose tolerance test (OGTT), were collected from October 1989 through February 1992. After excluding 56 non-Caucasians the cohort consisted of 2484 men and women. For the present study we excluded 612 subjects with missing information on morbidity during follow-up, because they did not provide written permission to accesses their medical records or they had moved out of Hoorn. Subjects with prevalent CVD/CHD and missing data on variables at baseline were excluded for the analysis, resulting in 1439 subjects. The Ethical Review Committee of the VU University Medical Centre approved the Hoorn Study and written informed consent was obtained from all participants.

### 2.2. Baseline examination

Serum ALT enzyme activity was measured according to the method of the International Federation of Clinical Chemistry from 1985, and expressed as U/L [12]. Since the reference range and cut-off values for ALT are controversial [13,14], we divided ALT into tertiles, instead of using a cut-off value to define abnormality. The OGTT was performed between 08.00 and 10.00 h and subjects were asked not to drink alcohol from 17 h and to fast (except for drinking water) from 22 h the evening before the measurements. Blood samples were collected before and 2 h after ingestion of the glucose load. Plasma glucose was measured with the glucose dehydrogenase method (Merck, Darmstadt, Germany). The glucose tolerance categories were defined according to the 1999 criteria of the World Health Organisation. Immuno-specific insulin was measured in serum with a double-antibody RIA (antibody SP21, Linco Research, St. Louis, USA). Insulin resistance was estimated by the homeostasis model assessment for insulin resistance (HOMA-IR), calculated as fasting insulin (in IU/L) × fasting glucose (mmol/L)/22.5 [15]. Glycated haemoglobin (HbA1c) was measured with ion exchange high performance liquid chromatography (Modular Diabetes Monitoring System, BioRad Lab, Veenendaal, The Netherlands; reference range 4.3–6.1%). Fasting triglycerides, total and HDL-cholesterol were determined in serum by enzymatic techniques (Boehringer Mannheim, Germany). LDL-cholesterol was calculated according to the Friedewald-formula, except in subjects with triglycerides >5.0 mmol/L. Seated systolic and diastolic blood pressure was measured at the right arm after a 5 min rest with a random-zero mercury sphygmomanometer (Hawksley-Gelman, Lancing, UK). The average of two measurements was used for the analyses. Weight and height were measured in subjects wearing light clothes only, and the body mass index (BMI) was calculated as weight divided by height squared (kg/m$^2$). Waist circumference was measured at the midpoint between the lowest rib and the iliac crest, hip circumference was measured at the widest level over the greater trochanters, and the mean value of two measurements was used in the analysis. Information on alcohol-intake was assessed by a validated semi-quantitative food frequency questionnaire [16]. Subjects were asked about their drinking habits and the weekly number of glasses of alcoholic drinks consumed. This information was converted to alcohol-intake (g/day) using a computerised version of the Dutch Food Composition Table. Physical activity was assessed by questionnaire as described before [11]. Information on history of cardiovascular disease was assessed by the Rose questionnaire. Smoking status was assessed by questionnaire and categorised as never, former and current smoker.

### 2.3. Follow-up

The registration of follow-up data of the Hoorn Study participants is regularly updated using the municipal register of the city of Hoorn providing information about the vital status of the participants. Information of causes of death and non-fatal events were obtained from medical records of general practitioners and from the local hospital. Causes of death were coded according to the ICD-9. CVD was defined as documented angina pectoris (chest pain followed by coronary artery bypass surgery or angioplasty or in the presence of >50 % stenosis or ECG changes, i.e. ST-segment elevation ≥1 mm or ST-segment depression ≥0.5 mm or positive exercise test), myocardial infarction (in the presence of at least two of the following: typical pain, elevated enzymes and/or ECG changes), congestive heart failure (in the presence of at least two of the following: shortness of breath, cardiomegaly, dilated neck veins or one of the former in the presence of oedema or tachycardia), stroke or transient ischaemic attack (sudden onset of symptoms, neurological symptoms or change of consciousness), peripheral disease

(both symptomatic and asymptomatic: by procedure or typical pain accompanied by stenosis or ankle arm blood pressure ratio <0.90 or positive vascular stress test). In fatal cases, CVD was defined with ICD codes 390–459 (diseases of the circulatory system) or 798 (sudden death, cause unknown), because sudden death in general is of CVD origin [17]. CHD was defined as documented myocardial infarction and angina pectoris, with the aforementioned criteria. Fatal CHD cases were defined with ICD coded 410–414 (ischaemic heart disease). Follow-up time was calculated as the time between the date of the baseline examination and the date of the first event or 1 January 2000.

### 2.4. Statistical analysis

Analyses were performed with SPSS 11.0.5 software. Subjects were categorised according to ALT tertiles. Differences between tertiles were tested with analysis of variance (ANOVA) for continuous variables and by the $\chi^2$-test for proportions. Hazard ratios and 95% confidence intervals of baseline ALT for all-cause mortality, incident CVD and CHD events were estimated from multivariable Cox proportional hazard analysis. The first models were adjusted for age and sex only. In the subsequent models we added the possible mediating or confounding factors one by one. In the final models, beside adjustments for age, sex, alcohol-intake, smoking and physical activity, we corrected for the traditional CVD risk factors, i.e. glucose tolerance status, systolic blood pressure, HbA1c, LDL-cholesterol, BMI (Table 2, model 2) as well as for the components of the metabolic syndrome, as defined by the Adult Treatment Panel III (NCEP) (Table 2, model 3), respectively. Two-sided $P$ values <0.05 were considered to indicate statistical significance.

## 3. Results

### 3.1. Baseline characteristics of the participants

The mean age of the 1439 participants (651 men and 788 women) was 60.9 (7.2) years. Table 1 shows the baseline characteristics of the participants divided into ALT tertiles. Subjects in the highest ALT tertile had a higher BMI, as well as waist and hip circumference. Also, they had higher fasting and 2 h post-load plasma glucose levels, fasting insulin levels, total cholesterol concentrations, and higher blood pressure. LDL-cholesterol tended to be different across the tertiles, whereas HbA1c was similar in all tertiles. The highest ALT tertile consisted of a higher number of subjects with impaired glucose metabolism and DM2.

### 3.2. Association of ALT and all-cause mortality, CVD and CHD events

During the 10-year follow-up, 174 of the total of 1439 subjects died, 355 CVD events occurred of which 75 where fatal

Table 1
Baseline characteristics of the participants ($N$ = 1439) stratified by ALT tertiles[a]

| Variable | Alanine aminotransferase activity (U/L) | | | |
|---|---|---|---|---|
| | 1 (mean (S.D.), median (interquartile range)) ($N$ = 551) | 2 (mean (S.D.), median (interquartile range) ($N$ = 420) | 3 (mean (S.D.), median (interquartile range)) ($N$ = 468) | $P$ |
| ALT (U/L)[b] | 12 (1–14) | 17 (15–20) | 26 (21–143) | |
| Age (years) | 62.9 (7.4) | 60.7 (7.1) | 59.7 (6.8) | <0.001 |
| Male (%) | 30.9 | 48.6 | 59.2 | <0.001 |
| Body mass index (kg/m$^2$) | 25.7 (3.4) | 26.1 (3.2) | 27.4 (3.3) | <0.001 |
| Waist (cm) | 87.0 (10.1) | 89.6 (10.3) | 94.2 (10.1) | <0.001 |
| Hip (cm) | 101.3 (6.7) | 100.9 (6.2) | 102.9 (6.4) | <0.001 |
| Fasting glucose (mmol/L) | 5.5 (1.1) | 5.7 (1.4) | 5.9 (1.5) | <0.001 |
| 2 h post-load glucose (mmol/L) | 5.6 (2.3) | 5.8 (3.0) | 6.5 (3.4) | <0.001 |
| Fasting insulin (pmol/L) | 79.8 (48.2) | 82.6 (49.9) | 96.1 (58.0) | <0.001 |
| HbA1c (%) | 5.4 (0.7) | 5.5 (0.8) | 5.5 (0.9) | 0.15 |
| Impaired glucose metabolism (%) | 12.0 | 15.0 | 21.6 | <0.001 |
| Type 2 diabetes mellitus (%) | 6.0 | 7.6 | 8.2 | 0.03 |
| Plasma lipids (mmol/L) | | | | |
| Total cholesterol | 6.6 (1.1) | 6.5 (1.1) | 6.7 (1.2) | <0.001 |
| LDL-cholesterol | 4.6 (1.0) | 4.5 (1.0) | 4.7 (1.1) | 0.051 |
| HDL-cholesterol | 1.38 (0.36) | 1.38 (0.37) | 1.30 (0.36) | <0.001 |
| Triglycerides | 1.4 (0.9) | 1.4 (0.7) | 1.8 (1.1) | <0.001 |
| Systolic BP (mmHg) | 133 (20) | 134 (20) | 137 (20) | <0.001 |
| Diastolic BP (mmHg) | 80 (11) | 82 (10) | 84 (10) | <0.01 |
| Smoking, current (%) | 35.2 | 27.9 | 26.3 | <0.001 |
| Alcohol-intake (g/day) | 2.2 (0–10) | 5.0 (0–15) | 10 (2–17) | <0.01 |

[a] Abbreviations: ALT, alanine aminotransferase; S.D., standard deviation; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
[b] Median (minimum–maximum value).

Table 2
Hazard ratios of the second and third compared to the first tertile of ALT in relation to all-cause mortality, CVD and CHD events[a]

| Model | Tertile | All-cause mortality, HR (95% CI), 174 events | CVD events, HR (95% CI), 355 events | CHD events, HR (95% CI), 129 events |
|---|---|---|---|---|
| Model 1[b] | Second | 0.74 (0.49–1.10) | 1.02 (0.78–1.33) | 0.94 (0.57–1.53) |
| | Third | 1.30 (0.92–1.83) | 1.40 (1.09–1.81) | 2.04 (1.35–3.10) |
| Model 1 + alcohol-intake | Second | 0.74 (0.50–1.10) | 1.02 (0.78–1.33) | 0.93 (0.57–1.52) |
| | Third | 1.30 (0.92–1.84) | 1.39 (1.08–1.80) | 2.03 (1.38–3.09) |
| Model 1 + BMI | Second | 0.72 (0.48–1.08) | 0.98 (0.75–1.28) | 0.90 (0.55–1.47) |
| | Third | 1.23 (0.85–1.76) | 1.27 (0.98–1.65) | 1.82 (1.18–2.81) |
| Model 1 + waist | Second | 0.70 (0.47–1.05) | 0.96 (0.73–1.26) | 0.89 (0.55–1.46) |
| | Third | 1.15 (0.80–1.65) | 1.24 (0.96–1.62) | 1.80 (1.17–2.77) |
| Model 1 + triglycerides | Second | 0.73 (0.49–1.09) | 1.02 (0.78–1.33) | 0.97 (0.57–1.53) |
| | Third | 1.24 (0.87–1.75) | 1.34 (1.04–1.74) | 1.97 (1.25–2.99) |
| Model 1 + total cholesterol | Second | 0.74 (0.49–1.09) | 1.02 (0.78–1.32) | 0.92 (0.57–1.51) |
| | Third | 1.27 (0.89–1.80) | 1.36 (1.05–1.75) | 1.89 (1.25–2.88) |
| Model 1 + fasting glucose | Second | 0.67 (0.45–1.01) | 0.96 (0.74–1.26) | 0.92 (0.56–1.51) |
| | Third | 1.21 (0.79–1.60) | 1.25 (0.97–1.62) | 1.98 (1.30–3.02) |
| Model 1 + fasting insulin | Second | 0.72 (0.48–1.07) | 1.03 (0.79–1.34) | 0.95 (0.58–1.56) |
| | Third | 1.29 (0.91–1.83) | 1.41 (1.09–1.82) | 2.06 (1.35–3.14) |
| Model 1 + HOMA-IR | Second | 0.71 (0.47–1.05) | 1.01 (0.78–1.32) | 0.95 (0.58–1.56) |
| | Third | 1.20 (0.85–1.72) | 1.34 (1.03–1.74) | 2.02 (1.32–3.10) |
| Model 2[c] | Second | 0.75 (0.50–1.12) | 0.99 (0.76–1.30) | 0.89 (0.54–1.47) |
| | Third | 1.21 (0.83–1.76) | 1.25 (0.96–1.64) | 1.75 (1.12–2.73) |
| Model 3[d] | Second | 0.71 (0.48–1.06) | 0.99 (0.76–1.20) | 0.93 (0.57–1.52) |
| | Third | 1.10 (0.77–1.61) | 1.22 (0.94–1.60) | 1.88 (1.21–2.92) |

[a] Abbreviations: ALT, alanine aminotransferase; CVD, cardiovascular disease; CHD, coronary heart disease; HR, hazard ratio; CI, confidence interval; BMI, body mass index; HOMA-IR, homeostasis model assessment for insulin resistance.
[b] Model 1: adjusted for age and sex.
[c] Model 2: adjusted for age, sex, alcohol-intake, smoking, physical activity, glucose tolerance status, systolic blood pressure, HbA1c, LDL-cholesterol, BMI.
[d] Model 3: adjusted for age, sex, alcohol-intake, smoking, physical activity, waist, triglycerides, systolic blood pressure, fasting glucose, HDL-cholesterol.

and 129 CHD events occurred of which 16 were fatal. Table 2 shows the hazard ratios of the third relative to the first ALT tertile for all-cause mortality, CVD and CHD events. The age- and sex-adjusted risk for all-cause mortality, CVD events and CHD events were 1.30 (0.92–1.83), 1.40 (1.09–1.81) and 2.04 (1.35–3.10), respectively, for the subjects in the upper ALT tertile compared to those in the first tertile. The association between ALT and all-cause mortality was not statistically significant and the association could be explained by the traditional CVD risk factors and/or the components of the metabolic syndrome (Table 2, models 2 and 3). The significant association of ALT with CVD events disappeared after adjustment for the classical CVD risk factors and/or the components of the metabolic syndrome (Table 2, models 2 and 3). Only the association of ALT and CHD events remained statistically significant after adjustment for CVD risk factors as well as for the features of the metabolic syndrome. The association of ALT and CHD events was explained by BMI, waist and to a lesser extent by total cholesterol and triglycerides. Fasting insulin and HOMA-IR did not substantially alter any of the associations presented (Table 2). Additional adjustment for baseline HDL in model 2 and additional adjustment for baseline LDL in model 3 (both HDL and LDL in one model) did not change the presented associations of ALT with CHD (data not shown).

### *3.3. ALT and CHD events: the effect of gender, alcohol-intake and diabetes*

In order to establish the robustness of the observed associations, we performed additional analyses, focusing on the role of gender, alcohol-intake and DM2. Stratified analysis for men and women separately yielded similar results (data not shown). Since DM2 carries a greater risk of CHD and CVD, and its association with elevated ALT, we subsequently excluded the 119 DM2 subjects from the analysis, which resulted in an attenuation of the association of ALT with CHD events. After adjustment for traditional CVD risk factors, the association became borderline significant (HR 1.65 [0.97–2.79]), whereas it remained significant after adjustment for the components of the metabolic syndrome (HR 1.78 [1.05–3.04]).

In order to detail the effect of alcohol on the association of ALT with CHD events, we excluded 232 subjects (77 women and 155 men) who had an average daily alcohol-intake of more than 20 g. Exclusion of these subjects attenuated the association, however, ALT remained a predictor for CHD after adjustment for classical CVD risk factors (HR 1.72 [1.02–2.90]) and for the components of the metabolic syndrome (HR 1.67 [0.99–2.80]).

## 4. Discussion

The main novel finding of this population-based study with well-documented characterisation and follow-up of the participants, was a significant prospective association of ALT with CHD events, independent of the traditional CVD risk factors, such as systolic blood pressure, HbA1c, LDL-cholesterol and BMI, and independent of the components of the NCEP-defined metabolic syndrome. In addition we confirmed the cross-sectional association of ALT, a marker of NAFLD, with components of the MetS, including measures of obesity, fasting glucose and lipids. No significant associations were found between ALT and CVD events or all-cause mortality, the latter observation is in accordance with a previous study that found no significant association of ALT with all-cause mortality [18]. The reason that we did not demonstrate a significant association of ALT and CVD events may be explained by that fact that the assessment of non-fatal incident CVD may be more subject to non-differential misclassification than non-fatal incident CHD events, thus leading to an underestimation of the true associations. Our study is, to our best knowledge, the first to demonstrate a prospective relation of ALT with CHD events and extends the results from cross-sectional studies demonstrating correlations of ALT with CHD risk factors, including the components of the MetS [9,19]. Bruckert et al. found that elevated ALT was associated with CHD risk factors, including elevated blood pressure, total cholesterol and triglyceride concentrations in 8501 hyperlipidaemic subjects [19]. We recently reported a positive association between ALT and endothelial dysfunction measured as brachial flow mediated vasodilatation. This association was independent of whole-body insulin sensitivity, indicating that other mechanisms besides insulin resistance, such as inflammation and/or oxidative stress might contribute to this association [9]. This is in accordance with the data from our present study, demonstrating that adjustment with HOMA-IR did not change the age- and sex-adjusted models to a significant extent. It should be noted that, although HOMA-IR is a validated surrogate measure for whole-body insulin sensitivity, it may not be a specific marker of hepatic insulin resistance. Targher et al. showed that healthy male volunteers with ultrasound documented NAFLD had an increased cIMT relative to those without NAFLD [20]. Since this relation was largely explained by the amount of visceral abdominal fat, adipose tissue derived adipocytokines were implicated as a link between fatty liver and atherosclerosis.

Taken together, three mechanisms, which all seem interrelated, may explain the association between ALT and the risk of coronary atherosclerosis. First, NALFD, often represented by its marker ALT, are linked with both hepatic and systemic insulin resistance and various components of the MetS [7,9]. As shown in recent reports form different populations, adults with the MetS are at increased risk of CHD, CVD and all-cause mortality [8,21]. Interestingly, however, adjustment for the components of the MetS did not blunt the association of ALT and CHD, indicating other contributing mechanisms. In addition, NAFLD is often referred to as the hepatic component of the MetS, and adjustment for the (other) components of the MetS may lead to an over adjustment in the association of ALT and CHD, since it is not yet fully clear whether the other components are cause, consequence or intermediate factors in the pathophysiology of NAFLD and CHD. Second, the low-grade systemic and hepatic inflammatory state may link elevated ALT to a high risk of CHD. In the normal liver, the production of cytokines is absent or minimal (insignificant), however, various stimuli such as reactive oxygen species, may induce the production of cytokines, such as tumour-necrosis factor-alpha and interleukin-6 [22], leading to inflammation and fibrosis. In addition to hepatic low-grade inflammation, adipose tissue compartments, both in healthy and in obese subjects are a major source of these cytokines. These cytokines may further amplify the inflammatory cascade by stimulating hepatic CRP production. A recent study demonstrated higher CRP levels in subjects with elevated ALT compared to subjects with normal ALT, even after adjustment for major confounding factors [23]. Indeed, CRP may be important in linking NAFLD to atherosclerosis since an elevated plasma CRP level is by now established as an independent risk factor of CHD and CVD events [24,25]. Third, elevation of ALT in response to fatty infiltration of the liver may similarly reflect excessive fat deposition in other organs such as the myocardium and the skeletal muscle. In these non-adipose tissues, intracellular triglyceride overload increases the pool of long-chain acyl Co-A, thus providing abundant substrate for non-oxidative metabolic pathways and the formation of toxic metabolites and also causing mitochondrial dysfunction and subsequent production of reactive oxygen species and ultimately leading to organ dysfunction and cell apoptosis [26].

A number of potential limitations of this study should be considered. The population consisted of Caucasian individuals aged 50–75, and caution should be exercised to generalise our findings to other populations. We excluded subjects with missing information on non-fatal disease because they did not give permission to access their hospital records. To study the possibility of selection bias, we repeated the analyses for all-cause mortality using the data from the entire original Hoorn Study cohort, without prevalent CVD/CHD ($n = 2007$), and the estimates were almost identical (data not shown). We used, similar to others, ALT levels as a marker of NAFLD instead of imaging or biopsies, the latter being regarded as the gold standard. Since serum ALT shows a strong correlation with biopsy proven fatty liver disease in patients and with liver triglyceride content, measured by MRI/MRS [27,28], its use as a marker of NAFLD in epidemiological studies seems appropriate. Although we addressed alcohol as an important confounder in our study, we could not account for other factors that might have caused a rise in ALT, such as viral hepatitis infections or hepatotoxic drugs. We did not assess the association of other liver enzymes such as AST and gamma-glutamyl transferase with outcome, as data on these markers were not available in the Hoorn Study cohort. Finally, we did

not measure CRP at baseline in the Hoorn Study, since low-grade inflammation may be a contributing mechanism in the association of ALT and CHD, future studies should address this issue.

The results of the present study show that ALT predicts CHD events, independent of traditional CVD risk factors and the components of the metabolic syndrome. Further studies are warranted to confirm these findings and to elucidate the pathophysiological mechanisms.

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